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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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R C van Dijk

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
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Pharmaceutical compositions and uses comprising Mucuna pruriens seed powder and extracts thereof in the treatment of neurological diseases

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Pharmaceutical compositions and uses comprising Mucuna pruriens seed powder and extracts thereof in the treatment of neurological diseases

The present invention provides pharmaceutical compositions comprising Mucuna pruriens seeds or one or more Mucuna pruriens seed components, substances, fractions or mixtures or substances obtained therefrom. Furthermore, the invention relates to the use of Mucuna pruriens seed powder or one or more Mucuna pruriens components, substances, fractions or mixtures or substances obtained therefrom for the preparation of a pharmaceutical composition for preventing, alleviating or treating neurological diseases. Additionally, the invention relates to the use of Mucuna pruriens seeds for the preparation of a pharmaceutical composition for neuroprotection or neurostimulation and to methods of preparing extracts of Mucuna pruriens which can be used for the preparation of a pharmaceutical composition for treating neurological diseases.

Several documents are cited throughout the text of this specification. The disclosure content of the documents cited herein (including any manufacture's specifications, instructions, etc.) is herewith incorporated by reference.

A large number of neurological and neurological degenerative disease are known, many of which are presently not curable. These diseases comprise medical conditions such as Parkinson's disease, Chorea Huntington, Hallervorder-Spatz disease, Alzheimer's disease, senile dementia, Creutzfeldt-Jakob disease, artheriosclerotic dementia, cerebral thrombangitis obliterans and many others. Parkinson's disease (PD) is a progressive movement and age-related disorder that is estimated to affect for example more than 500.000 persons in the United States, with as many as 50.000 new cases each year, at an estimated cost of 27 billion dollars annually. Usually PD begins in a person's late 50s or early 60s, it causes a progressive decline in movement control, affecting the ability to control initiation,

speed and smoothness of motion. Symptoms of PD are seen in up to 15% of those between the ages 65 to 74, and almost 30% of those between the ages of 75 and 84. PD is one of the best characterized diseases of the basal ganglia. The symptoms that come along with the disease are a rhythmical tremor at rest, a unique increase in muscle tone or rigidity that has often cogwheel- or ratchet-like characteristic, difficulty in the initiation of movement and paucity of spontaneous movements (akinesia), and slowness in the execution of movement (bradykinesia). In humans suffering from Parkinson's disease dopamine is missing or most drastically reduced in certain regions of the brain which are essentially needed, for example, for controlling the movement of the body. L-DOPA is metabolised within the body to dopamine which plays an outstanding role in the metabolism of the brain as neurotransmitter.

Beside the reduction of dopamine which coincides with the symptoms of the disease, it is also speculated that the destruction of dopamine-producing nerve cells especially those of the substantia nigra pars compacta in the mid brain (one of the principal movement control centers in the brain) contributes to the disease. This control center helps to refine movement patterns throughout the body. It was observed that the brains of patients with Parkinson's disease also have loss of nerve cells and depigmentation in the two pigmented loci of the brain stem: the substantia nigra and the locus ceruleus. Thereby, the severity of changes in the substantia nigra parallels the reduction of dopamine in the striatum. Because the pars compacta of the substantia nigra contains many of the dopaminergic nerve cell bodies in the brain, these observations suggest that the dopaminergic pathway from the substantia nigra to the striatum is disturbed in Parkinson's disease. However, the molecular mechanisms underlying PD are still under investigation and poorly understood.

From the above-mentioned findings that brains of PD patients have a drastically reduced dopamine level, it was reasoned that they might be helped if the amount of dopamine in the brain were restored to normal. Therefore, among others, L-3,4-hydroxyphenylalanine (L-DOPA), also known as levodopa, was administered intravenously to patients. L-DOPA, as the immediate precursor of dopamine is, in contrast to dopamine, capable of crossing the blood-brain barrier. After the

prolonged administration, a remarkable but brief remission in the patient's symptoms was observed which suggested an approach for the treatment of Parkinson's disease. However, this effect is generally associated with long term side effects and disease progression is not prevented. The reason for the strong side effect observed during treatment with levodopa is not known but suggests a toxic effect of said compound or its metabolites, including dopamine. Furthermore, it has been postulated that L-Dopa and its metabolite dopamine themselves have a toxic effect on neural-tissues and thus, besides alleviating the disease symptoms, may contribute to disease progression. It is important to note that Dopamine does not pass the blood-brain barrier in sufficient quantities, thus only a small percentage of L-Dopa reaches the brain after systemic administration. Moreover, L-Dopa is quickly metabolised peripherally, therefore high systemic L-Dopa doses are required to achieve the clinical effect (3 – 4 gr. L-Dopa/day). In view of the fact that immediate side-effects are directly related to L-Dopa peak plasma levels, L-Dopa was, in recent years, administered in combination therapy with other compounds such as decarboxylase and COMT (Catechol-amine-O-methyl-transferase) inhibitors to prevent periferal metabolisation. To prevent the metabolisation of dopamine in the brain, MAO (Mono-amine oxidase) inhibitors were also used. With these additives it was possible to reduce the daily required dose of L-Dopa to an average of about 600 mg/day. However, these additives were only partially capable of reducing the toxic side effects of the treatment with levodopa and could not prevent disease progression. Thus, there was an urgent need for an effective treatment of Parkinson's disease and other levodopa-sensitive neurological diseases which is not associated with or counteracting the side-effects of L-Dopa therapy including neurotoxicity.

Thus, the technical problem underlying the present invention was to provide means and methods for treating neurological diseases including Parkinson's disease.

The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a pharmaceutical composition comprising Mucuna pruriens seed powder or one or more components, substances, fractions or mixtures of substances obtained therefrom and a pharmaceutically acceptable diluent, excipient or carrier.

Mucuna pruriens is a plant of the family Leguminoseae and is indigenous to tropical countries like India and West Indies. It is an annual, climbing leguminous vine capable of growing to 6 m in length. The lanceolate leaves are alternate with three large, rhomboid-ovate leaflets. The flowers grow in racemes in 2 or 3 and are white to dark purple and hang in long racemes. Mucuna pruriens produces clusters of pods that are curved (4 to 8 cm long) and contain 2 to 6 seeds. The seeds vary in colour from black, white to mottled. The pods which are thick and leathery are covered with reddish-orange long stiff hairs that are readily dislodged and can cause intense irritation to the skin.

Since Mucuna pruriens and its use is so widespread that it is considered common fare from China to England, Iran to Spain, Africa to South America, it has a variety of common names like Nescafe, Cowage, Velvetbean, Fagiolo Di Rio Negro, Fogarate, Jeukerwt, Juckbohne, Nd, Pien Tou, Pois A Gatter, Pois Gratte, Swagupta, T'Ao Hung King, Kekara gatel or Rarawejah.

Velevetbean, a vigorous annual climbing legume, originally came from southern China and eastern India, where it was at one time widely cultivated as a green vegetable crop. The genus Mucuna, belonging to the Fabaceae family, covers perhaps 100 species of annual and perennial legumes, including the annual velvetbean.

According to Dr. Duke's Phytochemical and Ethnobotanical Databases at phytochemical Database, USDA-ARS-NGRL, Beltsville Agricultural Research Center, Beltsville, Maryland (*http://www.rain-tree.com/db/Mucuna-pruriens-phytochem.htm*) Mucuna pruriens contains many diverse Phytochemicals like 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolone, 5-hydroxytryptamine, 5-methoxy-n,n-dimethyltryptamine-n-oxide, 5-oxyindole-3-alkylamine, 6-methoxyharman, Alanine, Arachidic-acid, Arginine, Aspartic-acid,

Behenic-acid, Beta-carboline, Beta-sitosterol, Bufotenine, Choline, Cis-12,13-epoxyoctadec-trans-9-cis-acid, Cis-12,13-epoxyoctadec-trans-9-enoic-acid, Cystine, DOPA, Gallic-acid, Glutamic-acid, Glutathione, Glycine, Histidine, L-DOPA, Lecithin, Leucine, Linoleic-acid, Mucunadine, Mucunain, Mucunine, Myristic-acid, N,n-dimethyltryptamine, N,n-dimethyltryptamine-n-oxide, Nicotine, Oleic-acid, Palmitic-acid, Palmitoleic-acid, Phenyalanine, Phosphorus, Proline, Protein, Prurienidine, Prurienine, Saponins, Serine, Serotonin, Stearic-acid, Threonine, Tryptamine, Tyrosine, Valine, Vernolic-acid. Therefore, Mucuna pruriens finds traditionally use in a number of diseases and is commonly used as carminative, hypotensive & hypoglycemic agent. Moreover it is also used as anodyne, antidotal, aphrodisiac, diuretic, nervine, resolvent, rubefacient, and vermifuge; used for anasarca, asthma, cancer, cholera, cough, diarrhea, dogbite, dropsy, dysuria, insanity, mumps, pleuritis, ringworm, snakebite, sores, syphilis, tumors, and worms.

From phytochemistry point of view, the drug contains dimethyltryptamine alkaloids and related alkaloids, lecithin and tannins as well as L-DOPA, a precursor of the neural transmitter dopamine. All these compounds are known to exist in the seeds of Mucuna pruriens. Therefore, plants like Mucuna pruriens provide a natural source for drugs for Parkinson's disease since they contain, among many other phytochemicals, large amounts of levodopa (L-DOPA).

The term "therapeutically effective" means in an amount sufficient to prevent, treat or ameliorate a disease or the symptoms associated with a disease. The term "obtained" means isolated, extracted or otherwise taken or gained from the seed. The person skilled in the art knows various techniques for isolating or obtaining compounds from plants, some of which are described below.

The term "pharmaceutically acceptable" means approved by a national regulatory agency or by a generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol

solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

The terms "components", "substances", "fractions" or "mixtures of substances" all refer to compounds or mixtures of compounds isolated from Mucuna pruriens seeds. The term "isolated" refers to the process of obtaining or isolating the compound. The isolated component may initially be present in a crude extract of the seed, together with many other components of the seed. Later stages of the extraction process will yield fractions containing a reduced variety of components. This mixture of components may have similar physical or chemical properties. Further fractionation, however, will ultimately result in the complete isolation of a single molecular species which is the "isolated" component. The term "substance" as used herein refers to the isolated or pure component. However, methods such as solvent extraction generally result in a final fraction which contains minute amounts of contaminants. Preferably the substance is 100% pure, less preferably the substance is 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 80% or 70% pure. However, in certain cases also substances with a smaller degree of purity can be therapeutically effective. Therefore, the invention also refers to substances which are only 60%, 55% or even 50% pure.

The pharmaceutical composition, Mucunia pruriens seed powder or extracts, disclosed by the present invention allow long term L-Dopa treatment of neurological diseases, including Parkinson's Disease, in the absence of the short- and long term side effects observed in conventional treatment approaches. The term "Mucunia pruriens seed powder" relates to powder prepared from the seeds of Mucunia pruriens. Powder of Mucunia pruriens seeds can be prepared from dried beans which are freed from their cutular hairs by a brushing machine, then milled in a special mill for herbal drugs. The resulting powder is passed through a standard sieve No. 4 or 5, corresponding to a mesh width of 850 or 355 μm. The pharmaceutical composition and specific extracts of the present invention, not containing L-Dopa, can be applied as such for the treatment of neurodegenerative diseases in general, or in combination with isolated L-Dopa if required.

Conventional L-Dopa therapy requires a gradual increase of the effective dose over time resulting of progression of disease and/or the neurotoxic effects of L-Dopa or dopamine with an increase of toxic reactions and, over time, the appearance of dyskinesia, increasing in severity with dose. In clinical experiences with Mucuna prurience seed preparations these negative phenomena have not been observed in that for the effective treatment of Parkinson's, the dose of Mucuna pruriens derived L-Dopa remained relatively stable over longer periods of time, and in that dyskinesia, even in patients with with pre-existing dyskinesia following long term therapy with conventional L-Dopa preparations, appeared to be less in occurrence and severity.

The present invention represents a rationale for this experience. Surprisingly, even in cases of relatively high levels of L-Dopa in blood following Mucuna pruriens administration, no immediate toxic effects were encountered normally to be expected with such levels following administration of conventional preparations.

In a preferred embodiment, the components, substances, fractions or mixtures of substances are extracted from Mucuna pruriens seeds.

The term "extraction" refers to the process of obtaining or isolating a compound from Mucuna pruriens seeds. The person skilled in the art knows of various extraction techniques all of which rely on the physical properties of the compounds to be

isolated. Extraction, as used herein, relates to the separation of medicinally active portions of Mucuna pruriens from the inactive or inert components through the use of selective solvents. The person skilled in the art knows that the term "extraction" comprises maceration, percolation, digestion, infusion and decoction. Many extraction methods contain one or more steps of mechanical treatment of the seed which is usually an initial step that may be followed by a filtration, a washing and/or a drying step. The extraction protocol may be composed of several extraction steps, resulting in the generation of one or more fractions containing various concentrations of the therapeutically active compound. However, methods such as solvent extraction generally result in a final fraction which contains minute amounts of contaminants. Preferably the substance is 100% pure, less preferably the substance is at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 80% or 70% pure. However, in certain cases also substances with a smaller degree of purity can be therapeutically effective. Therefore, the invention also refers to substances which are only 60%, 55% or even 50% pure. When the extraction protocol comprises more than one extraction step, the extraction steps may be followed or preceded by additional steps of pre-treatment. These pre-treatments may be important in order to optimise the quality and/or quantity of the next extraction process and may include steps such as mixing, heat-treatment, addition of chemical compounds, filtration, distillation.

In a preferred embodiment, the pharmaceutical composition comprise bipolar-lipophilic molecules obtained by extraction of Mucuna pruriens.

Solvent extraction relies on differential solvent solubility of the seed components. Polar solvents will tend to extract water-soluble compounds, non-polar solvents will tend to extract lipophilic compounds, while amphiphilic solvents can extract lipophilic as well as water-soluble compounds. The person skilled in the art knows of various solvents that can be used in an extraction process. Preferably the solvent used is a single solvent selected from the group consisting of water, hexane, acetone, ethanol, chloroform, ethylacetate, diclormethane and petrolether. However, also preferred are mixtures of two or more solvents. The solvent may additionally contain compounds such as enzyme inhibitors including phosphatase inhibitors or protease

inhibitors, reducing or oxidizing agents or the like including DTT, GSH, ascorbic acid or SO_2-gas, chelating agents including EGTA or EDTA, and mono- or divalent ions including Mg^{2+}, Ca^{2+}, Na^+, Li^+, Cl^-, SO_4^{2}, K^+, NO_3. These additional compounds present in the solvent or added after extraction may be important to preserve the physical state of the therapeutically active compound. Another preferred embodiment is extraction in the presence of gases such as nitrogen or argon which may also be important for controlling the oxidative state of the extract.

Physical parameters such as pressure or temperature may play an important role for extraction procedures since they can have a strong impact on the state of aggregation of the seed components or of the solvent. High pressure, e.g., results in liquefaction of carbon dioxide and other gases and may have a strong impact on the result of the extraction process. Carbon dioxide, e.g. is known to be an excellent solvent at hypercritical conditions. Methods of extracting plant material, including methods of extraction with hypercritical CO_2, are known in the art (Verdichtete Gase zur Extraktion und Raffinierung, E. Stahl, Springer Verlag, Heidelberg, Berlin, 1986). Accordingly, the extraction process of the present invention may be performed at low pressure, i.e. at low pressure: < 200 bar, intermediate pressure: 200 – 300 bar, high pressure:> 300 bar. Similarly, the temperature during the extraction process can be important for the yield of the extraction process. Accordingly the present invention can be performed at low temperature, i.e. between 0°C and 10°C, at an intermediate temperature, i.e. between 10°C and 40°C or at high temperature, i.e. between 40°C and 100°C. [Please modify these ranges if appropriate].

Accordingly, the present invention relates in a preferred embodiment to a method for the preparation of extracts or extract fractions of Mucuna pruriens, comprising extracting the seed of Mucuna pruriens with CO_2 or mixtures from CO_2 and butane, propane or other gases under supercritical conditions or different pressures and temperatures, to obtain purification and selection of substances or fractionation of Mucuna pruriens extracts.

In a preferred embodiment of the present invention, Mucuna pruriens seeds or seed powder is extracted twice with acetone. The remaining material is further extracted with n-propanol.

In another preferred embodiment of the present invention, Mucuna pruriens seeds or seed powder is extracted at least one time with a 1:1 mixture of water and ethanol. The extraction process can be performed in the presence or absence of ascorbic acid. In another preferred embodiment of the present invention, Mucuna pruriens seeds or seed powder is initially extracted by water, the resulting extract is further fractionated by ethanol precipitation.

In a more preferred embodiment, the components, substances, fractions or mixtures of substances are extracted from Mucuna pruriens seeds by using bipolar-lipophilic solvent molecules such as acetone, DMSO or dimethylformamide which extract liphophilic and a great part of polar of hydrophilic substances from the plant material. These solvents are sometimes also designated simply as bipolar. Other extraction methods are staggered procedures starting with organic solvents followed by polar solvents or vice versa. Very often also water-alcohol, alcohol-acetone or acetone – hexan solvent mixtures are used to extract lipophilic and hydrophilic constitutents in one extraction operation. Preferably the bipolar-lipophilic solvent is selected from the group consisting of acetone, DMSO or dimethylformamide. However, this list is non-limiting and the person skilled in the art knows of many other bipolar-lipophilic molecules which might be used in the extraction process.

In another preferred embodiment, the pharmaceutical composition is formulated as an infusion, an injection solution, a gelatin-capsule, a tablet or a controlled relase tablet. Many delivery systems are known to the person skilled in the art and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal

mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment. The compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.). The compound or composition can be delivered in a controlled release system, including the use of a pump (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). The above-mentioned controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

The present invention also provides the use of Mucuna pruriens seeds or one or more components, fractions or mixtures of substances obtained or extracted from Mucuna pruriens for the preparation of a pharmaceutical composition for neuroprotection or neurostimulation.

The term "neuroprotection" means protection of nerve tissues. Furthermore, neuroprotection is the protection of nerve cells and their function against endogene or exogene physical or chemical factors negatively influencing the metabolism, survival or function of nerve cells such as for example heat application, Roentgen radiation, ischaemia, neurotoxins, oxidants, intoxications including heavy metals,

infections and sequelae of vaccinations, systemic metabolic diseases and disturbances in endocrine or electrolyte homeostasis.

The term "neurostimulation" means stimulation of nerve tissues. Moreover, neurostimulation is the improvement, enhancement or restoration of function of nerve cell tissues, both centrally and peripherally, through stimulation of cellular growth or nerve cell activity, both with respect to signal conduction and transduction, by physical means such as for example electro-stimulation or chemical-pharmacological means. The neuroprotective effect of Mucuna pruriens is registered by in vitro measurements of the survival and growth rate of mesencephalic or motor-neurons after pretreatment with Mucuna extract and exposure to a damaging (oxidative, toxic) stress agents.

The present invention also provides the use of one or more Mucuna pruriens components, fractions or mixtures of substances obtained or extracted from Mucuna pruriens for the preparation of a pharmaceutical composition for preventing, alleviating or treating neurological diseases. The term "neurological disease" includes diseases such as Parkinson disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (aALS), motoneuron disease.

A preferred embodiment of the invention the use relates to neurological degenerative diseases and comprises a large number of diseases known to the person skilled in the art. According to the present invention, neurodegenerative or neurological degenerative diseases fall into one of the following groups A to D:

A: Degenerative and Heredodegenerative Diseases (nerve tissue atrophy is primair). This group includes, but is not limited to: Parkinson's disease, Chorea Huntington, Hallervorder-Spatz disease, Alzheimer's disease, senile dementia, Creutzfeldt-Jakob disease, artheriosclerotic dementia, cerebral thrombangitis obliterans.

B: Metabolic and nutritional Disorders (secondair to systemic disorder). This group includes, but is not limited to: disturbances in lipoid metabolism (a.o.

Gaucher,Niemann-Pick, Tay-Sachs, Hurler, Refsum), Leukodystrophien, disturbances in aminoacid, carbohydrate metabolism, hepatolenticular degeneration, etc. deficiencies such as Vitamine B12 deficiency or folic acid deficiency.

C: Systemic diseases, endocrine disturbances and autoimmune reactions afflicting the nervous system: This group includes, but is not limited to: hypothyreose, hypo- and hyperparathyreoidismus, Collageen diseases, systemic lupus erythematodes, sarcoidosis, leukoencephalopathy, demyelisation.

D: Nervous tissue damage by various endogenic and exogenic factors: This group includes, but is not limited to: ischemia, trauma's, physical noxen, intoxications with metals (mercury, lead, aluminum, etc) and neurotoxins, alcohol abuse, sequelae of infection and vaccination.

Neurological degenerative diseases, as used herein, that may be prevented, treated or alleviated are diseases characterized by the formation of nervous system lesions. These lesions include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis

(ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis. Neurological degenerative disease associated with the formation of lesions and/or behavioral disorders include, but are not limited to, Alzheimers Disease, Parkinsons Disease, Huntingtons Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception.

In a more preferred embodiment the neurological degenerative disease is selected from the group the group of degenerative and heredodegenerative diseases including Parkinson's disease, Chorea Huntington, Hallervorder-Spatz disease, Alzheimer's disease, senile dementia, Creutzfeldt-Jakob disease, artheriosclerotic dementia, cerebral thrombangitis obliterans or other diseases, according to any one of the diseases mentioned under group A to D of neurodegenerative diseases, which can be caused by exogenic or endogenic factors. The term "endogenic factor" means originating from within an organism, the term "exogenic factor" means originating from outside the organism.

Yet another more preferred embodiment relates to a neurological degenerative disease which is Parkinson's disease.

Still another preferred embodiment relates to the use of Mucuna pruriens seed powder or one or more Mucuna pruriens components, fractions or mixtures of substances obtained or extracted from Mucuna pruriens for the preparation of a pharmaceutical composition for preventing, alleviating or treating a neurological degenerative disease, wherein said components, fractions or mixtures of substances obtained or extracted from Mucuna pruriens can be or contain any chemical entity contained in Mucuna pruriens. However, preferably said components, fractions or mixtures of substances are selected from the group consisting of alkaloids, proteins, peptides, polysaccharides, glycosides, glycoproteins, sterols, phytochemicals like 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinolone, 5-hydroxytryptamine, 5-methoxy-n,n-dimethyltryptamine-n-oxide, 5-oxyindole-3-alkylamine, 6-methoxyharman, Alanine, Arachidic-acid, Arginine, Aspartic-acid, Behenic-acid, Beta-carboline, Beta-sitosterol, Bufotenine, Choline, Cis-12,13-epoxyoctadec-trans-9-cis-acid, Cis-12,13-epoxyoctadec-trans-9-enoic-acid, Cystine, DOPA, Gallic-acid, Glutamic-acid, Glutathione, Glycine, Histidine, L-DOPA, Lecithin, Leucine, Linoleic-acid, Mucunadine, Mucunain, Mucunine, Myristic-acid, N,n-dimethyltryptamine, N,n-dimethyltryptamine-n-oxide, Nicotine, Oleic-acid, Palmitic-acid, Palmitoleic-acid, Phenyalanine, Phosphorus, Proline, Protein, Prurienidine, Prurienine, Saponins, Serine, Serotonin, Stearic-acid, Threonine, Tryptamine, Tyrosine, Valine, Vernolic-acid or phosphatides such as phosphatidylcholin, phosphatidylethanolamine, phosphatidylserin, phosphatidylinositol. Preferred alkaloids include L-3-Carboxy-6,7-dhihydroxy-1,2,3,4-tetrahydroisoquinoline, L-3-Carboxy 7,8-dhihydroxy-1,1-dimethyl, 1, 2, 3, 4 – tetra- hydroisoquinoline, L-3-Carboxy-6,7-dihydroxy-1,1-dimethyl-1, 2, 3, 4 – tetra-hydroisoquinoline, L-3-Carboxy-6,7-dihydroxy-1 β-methyl-1, 2, 3, 4-tetraisoquinoline and 1-methy-3-carbox-6,7-dihydroxy-1, 2, 3, 4-tetrahydroiso-quinoline. More preferably said components, fractions or mixtures of substances contain L-Dopa and one or more components contained in Mucuna pruriens. Another preferred embodiment of the invention relates the use of components, substances, fractions or mixtures of substances isolated from Mucuna pruriens which do not contain a pharmaceutically effective amount or only traces of L-Dopa.

Another preferred embodiment of the invention relates to the use of alcohols and/or mixtures thereof, used for the extraction of Mucuna pruriens components, fractions or mixtures of substances, wherein the alcohol is selected from the group consisting of hexanol, ethanol, methanol, isopropanol, n-butanol and propanol.

A further preferred embodiment relates to the use of organic solvents and/or mixtures thereof, used for the extraction of Mucuna pruriens components, fractions or mixtures of substances, wherein the organic solvent is selected from the group consisting of chloroform, CO_2, hypercritical CO_2, ether, DMSO, hexane, ethylacetate, dichlormethane and acetone.

Yet another preferred embodiment relates to the use of polar solvents and/or mixtures thereof, used for the extraction of Mucuna pruriens components, fractions or mixtures of substances, wherein the polar solvent is selected from the group consisting of water, ethanol, methanol, propanol and isopropanol.

A more preferred embodiment relates to the use of a 1:1 mixture of water and ethanol containing 0.5% ascorbic acid [%w/w]. Preferably this step is preceded by an initial extraction step with n-hexane followed by an extraction step with acetone. However, the invention also relates to the use of mixtures, wherein the ethanol concentration is preferably between 10% and 75%, more preferred between 20% and 65%, even more preferred between 30 % and 60% and most preferred 50%. Preferably said mixture contains between 0.01% and 2% ascorbic acid, more preferred between 0,1% and1,2%, even more preferred between 0,3% and 0,9% and most preferred 0,5% ascorbic acid. However, in some cases it may be advantageous to add no ascorbic acid to the extraction solution.

Another preferred embodiment relates to the use of one or more solvents or mixtures of the solvents for the extraction process. According to this embodiment, two or more solvents selected from the group of alcohols, organic solvents and polar solvents are used for the extraction process.

Still another embodiment relates to fractionated extraction.

The present invention also provides a method of preparing extracts or extract-fractions of Mucuna pruriens comprising (a) extracting seeds of Mucuna pruriens with n-hexane to provide a first extract solution; (b) filtering the first extract solution; (c) extracting the filter retentate of (b) with acetone to provide a second extract solution; (d) filtering the second extract solution; (e) extracting the filter retentate of (d) with a 1:1 mixture of water and ethanol containing 0,5% ascorbic acid to provide third extract solution; (f) filtering the third extract solution; (g) repeating at least four times the extraction procedure of (e) with the retentate obtained by (f); and (h) concentrating the pooled extract solutions. However, the invention also relates to methods comprising additional filtration, washing or extraction steps. The term "filtration" means filtration by passing through a filter. The term "washing" means to wash the filtered residue on a filter with the mentioned solvent. The term "concentrating" means evaporation at low temperature (40-75°C) and at normal or under reduced pressure. Water extract could alternatively be freeze-dried.

Furthermore, the invention also relates to a method wherein hexane in the initial step and/or acetone in the second extraction step is replaced by at least one other organic solvent as defined above. Furthermore, the invention also relates to a method wherein the ethanol content and/or the content of ascorbic acid are modified. In particular, the ethanol concentration may be modified to any concentration between 10% and 75%, more preferred between 20% and 65%, even more preferred between 30 % and 60% and most preferred between 45% and 55%. Preferably said mixture contains between 0.01% and 2% ascorbic acid, more preferred between 0,1% and 1,2%, even more preferred between 0,3% and 0,9% and most preferred between 0,45% and 5,5% ascorbic acid. However, in some cases it may be advantageous to add no ascorbic acid to the extraction solution. Furthermore, this method of the invention allows replacement of ethanol with other alcoholic compounds selected from the group consisting of propanol, isopropanol and methanol. The method for preparing extracts or extract-fractions of Mucuna pruriens comprises repeating the extraction procedure of step (e) on the retenate of step (f). Nevertheless, in some cases it may be advantageous to eliminate this step

from the method or to repeat this step at least once, at least twice, at least three times, at least four times, at least five times or up to ten times.

The present invention also provides a method of preparing extracts or extract-fractions of Mucuna pruriens comprising (a) extracting seeds of Mucuna pruriens with an alcohol, wherein the alcohol is (i) methanol, ethanol and/or propanol to provide a first extract solution; (b) filtering the first extract solution; (c) repeating at least two times the extraction procedure of (a) with the retentate obtained by (b); and concentrating the pooled extract solutions. Preferably the first extract solution consists of 10-100% alcohol, more preferably of 30-100% alcohol, even more preferably of 70-100% alcohol, still more preferably of 90-100% alcohol and most preferably of 99-100% alcohol. This method for preparing extracts or extract-fractions of Mucuna pruriens comprises repeating the extraction procedure of step (a) with the retenate of step (b). In some cases it may be advantageous to eliminate this step from the method or to repeat this step at least once, at least twice, at least three times, at least four times, at least five times or up to ten times. Furthermore, in some cases it may be advantageous to add organic solvents such as e.g. Dimethyl Sulfoxide (DMSO) or water.

In a preferred embodiment the method further comprises solubilizing said extract or extract-fractions of Mucuna pruriens in a solvent comprising DMSO and/or distilled water. Resolubilization may be supported by heat treatment of the extract in the presence of the solubilizing agent, appropriate conditions can easily established by the person skilled in the art.

The invention also relates to a method for the preparation of extracts or extract fractions of Mucuna pruriens, comprising extracting the seed of Mucuna pruriens with CO_2 or mixtures from CO_2 and butane, propane or other gases under supercritical conditions or different pressures and temperatures, to obtain purification and selection of substances or fractionation of Mucuna pruriens extracts.

The present invention also provides the use of Mucuna pruriens seeds or seed powder, as well as extracts or extract fractions of Mucuna pruriens, obtainable by

the methods of the invention, for the preparation of a pharmaceutical composition for treating neuronal diseases. The term "obtainable" or "obtained" means produced, isolated or extracted by any of the methods of the invention.

A preferred embodiment of the invention relates to the use of extracts or extract-fractions or of extracted components, substances or mixtures of substances wherein Mucuna pruriens is used in comminuted form, as granules, powder, precipitate, fraction, extract, dried extract and/or exudates, preferably as extract.

Another preferred embodiment of the invention relates to the use of Mucuna pruriens seeds or seed powder or extracts of Mucuna pruriens or extract-fractions or of extracted components, substances or mixtures of substances, wherein said Mucuna pruriens components, substances, fractions or mixtures of substances obtained therefrom are used in combination with one or more other active agents. The term "active agent" as used herein relates to therapeutic agents or products clinically used or to be used in future, such as components contained in amino acid fractions with or without L-DOPA, isoquinoline alcaloid fractions, polysaccharide or glycoprotein fractions, phosphatides, fatty acid fractions. for the treatment of neurological diseases in which a combination of Mucuna pruriens components, substances, fractions or mixtures of substances with the active agent could be of clinical benefit.

Mucuna pruriens components, substances, fractions or mixtures of substances will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with said Mucuna pruriens extract alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

Pharmaceutical compositions containing the extracts of the invention are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or

formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The extract is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. NO: 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers*, 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981), and Langer, *Chem. Tech.*, 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomally entrapped extracts. Liposomes containing the Mucuna pruriens extract are prepared by methods known by the person skilled in the art. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

For parenteral administration, in one embodiment, the extract is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the therapeutical effectiveness of Mucuna pruriens extracts.

Generally, the formulations are prepared by contacting the Mucuna pruriens extract uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate may also be useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients

at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Mucuna pruriens extract is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8.

Any Mucuna pruriens extract to be used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic Mucuna pruriens compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Mucuna pruriens ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. The infusion solution is prepared by reconstituting the lyophilized Mucuna pruriens extract using bacteriostatic Water-for-Injection.

Another preferred embodiment of the invention relates to the use of extracts or extract-fractions or of extracted components, substances or mixtures of substances wherein the Mucuna pruriens components, substances, fractions or mixtures of substances are formulated as infusion solution, injection solution, for oral forms of application, as a therapeutic pack, a granulate, a food supplement or in form of clysters.

Yet another preferred embodiment of the invention relates to the use of extracts or extract-fractions or of extracted components, substances or mixtures of substances in oral, topical and/or parenteral applications.

Finally, the invention also provides a pharmaceutical pack or kit comprising one or more containers filled with Mucuna pruriens components, substances, fractions or mixtures of substances or the pharmaceutical compositions of the invention.. Associated with such container(s) can be a notice in the form prescribed by a national health authority regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Mucuna pruriens extracts of the present invention may be employed in conjunction with other therapeutic compounds.

The invention is illustrated by the following examples:

Material and Methods:

(1) Experimental Design

Thirteen (13) different extracts of *Mucana Pruriens* were tested on the survival of primary cell cultures of mesencephalic neurons. Cell cultures were prepared from embryonic rodent mesencephalon on the 14th day of gestation. Three or four concentrations of each extract were tested in triplicate in each paradigm, according to a previously published method (Mytilineou et al 1997, 1998). All experiments have been performed in a blinded fashion.

Extracts of *Mucana Pruriens* were tested for their effect on the following conditions:

(I) Survival of cultured dopaminergic neurons

(II) Survival of cultured dopamine neurons following exposure to:

(a) depletion of GSH by buthionine sulfoximine (3 concentrations)

(b) exposure to MPP^+ (3 concentrations)

(2) Analysis of Data

The effect of extracts of *Mucana Pruriens* on the survival of dopamine neurons were assessed by measuring:

(a) the uptake of dopamine as a measure of the number of dopamine terminals and an index of the number of surviving dopamine neurons.

(b) MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromid) reduction as a measure of cell survival

(c) LDH (lactate dehydrogenase) release as a measure of number of survival cells

These techniques are well known to the person skilled in the art and have been published previously (Mytilineou et al 1997, 1998).

(3) Miscellaneous Material

Pregnant rats are purchased from Taconic Farms (Germantown, NY). Minimum essential medium (MEM) is obtained from GIBCO (Grand Isand, NY), horse serum from Gemini (Calabasas, CA), and NU serum from Becton Dickinson (Bedford, MA).

The Vectastain ABC Kit is form Vector Laboratories (Burlingame, CA). Other chemicals are purchased form Sigma (St. Louis, MO).

(4) Cell Culture

Mesencehalic cultures are prepared from rat embryos on gestational day 14 as previously described (Mytilineou et al 1993). Dissociated cells are plated on poly-l-ornithine (0.1 mg/ml)-coated dishes (35 mm in diameter, Falcon) at a density of 10^5 cells/cm^2. The feeding medium consists of MEM with 30 mM glucose, 2 mM glutamine, 10% horse serum and 10% NU serum (which contains 25% fetal calf serum and other additions).

(5) Assay for Dopamine uptake

[^{3}H]DA uptake is determined as previously described (Hou et al. 1996). In brief, cultures are washed twice to remove residual drugs and incubated for 15 min in [^{3}H]DA (0.5 Ci/ml, 21.4 Ci/mmol). After two rinses and a 5 min incubation with fresh buffer, the accumulated [^{3}H]DA is extracted in 1ml of 95% ethanol, added to 10 ml of Exoscint A and counted in a scintillation spectrometer.

(6) MTT assay

Cell viability was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromid) reduction assay, as described previously (Han et al., 1996). In brief, 50 µl of a 5 mg/ml solution of MTT was added to each cell culture well containing 0.5 ml medium. After 3 hours incubation at 37°C the medium was removed carefully and the formazan crystals formed were dissolved in 1 ml isopropyl alcohol by gently shaking of the plate. Absorbance was determined at 570 nm in a microplate reader.

(7) LDH assay

A modification of the method by Bergmeyer et al. (1963) was used to determine LDH activity in the culture medium and the cells. Culture medium was collected, centrifuged to remove debris and frozen at -80°C until assay. Cells were collected in 1.0 ml of 50 mM potassium phosphate buffer at pH 7.2, sonicated in the cold for 10

s and frozen at -80°C. 100µl of supernatant and 100µl of NADH (1.2 mg/ml H_2O stock) were added to 800µl of buffer and the samples were vortex-mixed. 250µl aliquots (triplicates) were placed into 96-well plates at room temperature and the reaction was initiated by addition of 25µl of sodium pyruvate (0.35 mg/ml H_2O stock). The rate of disappearance of NADH was measured at 340 nm on a plate reader (Spectramax™, Molecular Devices Corporation, Sunnyvale CA).

(8) Statistical assessment

For multiple comparisons, statistical analysis were carried out using an ANOVA followed by Tukey's or Dunnett's test. Significance between groups was tested with an independent two-tailed t-test.

(9) Composition and properties of extracts

1. **M-HX1299 (50 mg)**

200 g pulverised seed material of *Mucana pruriens* were shaken at room temperature in 200 ml n-hexane for 18 hours. After filtration, the material was further washed with 100 ml n-hexane and filtered. The filtrates were collected and the solvent distilled off to obtain a yellowish oil.

2. **M-AC1299 (50 mg)**

The residue of the n-hexane extraction (was above), was shaken for 18 hours at room temperature in acetone (200 ml) and filtered. The residue was extracted once more with 200 ml acetone by further shaking for 18 hours and filtered. The residue was washed with acetone (100 ml) and filtered. After pooling, the filtrates were evaporated by distillation under reduced pressure yielding a yellowish mass.

3. **M-W-EL1299 (50 mg)**

The residue (obtained from the above extractions, ca. 96 g) was shaken for 18 hours at room temperature in 500 ml of a mixture of water- EtOH, 1:1 with 0.5% ascorbic acid. The solvent was filtered and concentrated under reduced pressure at a temperature of 35°C. Above extraction procedure was repeated

four times. After concentration, the filtrates were collected and the solvent removed under vacuum to get the solid mass.

4. **M-CH1299 (50 mg):**

100 g of the pulverised seed s of *M. pruriens* were shaken for 18 hours at room temperature in EtOH (100 ml). After filtration, the residue was again shaken for 18 hours and filtered. The process was repeated for a total of four extractions. The filtrates were concentrated and pooled together to get the extract.

5. **M-EL0100 (50 mg):**

20 g of the pulverised seeds of *M. pruriens* were shaken for 18 hours at room temperature in EtOH (100 ml). After filtration, the residue was again shaken for 18 hours and filtered. The process was repeated for a total fo four extractions. The filtrates were concentrated and pooled together to get the extract.

6. **M-W0100 (50 mg):**

The residue obtained from the ethanol extraction, as stated above, was further shaken for 18 hours at room temperature in demineralised water and filtered. The extraction was repeated for three times more. The filtrates were pooled together and water distilled off, after passing SO_2 to prevent the oxidation of L-DOPA. The solid sticky extract was thus obtained.

7. **M-ML0100 (50 mg):**

10 g of the pulverised seeds of *M. pruriens* were shaken for 18 hours at room temperature in methanol (50 ml) and filtered. The extraction of the residue was followed two more times with methanol and filtered. The filtrates were pooled together and the solvent distilled off to yield the semi-solid mass.

8. **M-BL0100 (50 mg):**

10 g of the pulverised seeds of *M. pruriens* were shaken for 18 hours at room temperature in n-butanol (50 ml) and filtered. The extraction of the residue was followed two more times with n-butanol and filtered. The filtrates were

pooled together and the solvent distilled off. The oily extract was thus obtained.

9. M-PL0100 (50 mg):

10 g of the pulverised seeds of *M. pruriens* were shaken for 18 hours at room temperature in n-propanol (50 ml) and filtered. The extraction of the residue was again followed two more times with n-propanol and filtered. The filtrates were pooled together and the solvent distilled off to get an oily mass.

10. M-ACPL0800

The M. *pruriens* seed powder was defatted twice for 18 hrs at room temperature with acetone by shaking the powder each with 200 ml acetone, the combined solvent evaporated at reduced temperature and the residue extracted for 18hrs at room temperature with 500 ml n-propanol and then evaporated to dryness. This extract contains traces (negligible) amounts of L-DOPA (in TLC detectable after enrichment only).

11. MWEL0700

The M. *pruriens* seed powder was not defatted with acetone, but directly extracted with a 1 : 1 mixture of water: ethanol without ascorbic acid addition by shaking 100 g powder with 500 ml of this solvent mixture for 18 hrs at room temperature. The solvent was evaporated at reduced temperatur to dryness. The extract contains L-DOPA in traces only.

SOLUBILITY OF THE EXTRACTS

1.	M-HX1299:	6.5 mg in 0.5 ml DMSO + 0.5 ml dist. water
2.	M-AC1299:	2.0 mg in 6 drops of DMSO + 0.2 ml dist. water
3.	M-W-EL1299:	5.0 mg in 0.1 ml dist. water
4.	M-CH1299:	10.0 mg in 0.3 ml DMSO + 0.8 ml dist. water
5.	M-EL0100:	3.0 mg in 2 drops DMSO +1.0 ml dist. water
6.	M-W0100:	7.0 mg in 0.4 ml warm (60°C) dist. water
7.	M-ML0100:	4.0 mg in 0.3 ml dist. water
8.	M-BL0100:	7.0 mg in 4 drops DMSO + 0.4 ml dist. water
9.	M-PL0100:	5.0 mg in 4 drops DMSO + 0.4 ml dist. water

L-DOPA COMPOSITION OF THE EXTRACTS

1.	M-HX1299:	No L-DOPA content.
2.	M-AC1299:	No L-DOPA content.
3.	M-W-EL1299:	High L-DOPA content.
4.	M-CH1299:	No L-DOPA content.
5.	M-EL0100:	Moderate L-DOPA content.
6.	M-W0100:	High L-DOPA content.
7.	M-ML0100:	Moderately high L-DOPA content.
8.	M-BL0100:	Moderate L-DOPA content.
9.	M-PL0100:	Trace of L-DOPA content.
10.	M-ACPL0800	Trace of L-DOPA content.
11.	MWEL0700	Trace of L-DOPA content.

Example 1: Extraction procedure of Mucuna pruriens seeds, fractionated extraction

Mucuna Pruriens extracts were generated by performing steps 1 to 7 of the following protocol:

Extraction procedures of *Mucuna pruriens* seeds (batch no. MU 99001)

1. Hexane extract (no. M-HX1299):
200 g pulverised seed material of *Mucuna pruriens* were shaken at room temperature in 200 ml n-hexane for 18 hrs. After filtration, the material was further washed with 100 ml n-hexane and filtered. The filtrates were collected and the solvent distilled off to obtain a yellowish oily liquid (5.5 g) in a yield of 2.75% (w/w). The 50 mg extract gave clear solution in 1 ml DMSO (5% v/v).

2. Acetone extract (no. M-AC1299):
The residue of the n-hexane extraction, was shaken for 18 hrs. at room temperature in acetone (200 ml) and filtered. The residue was extracted once more with 200 ml acetone by further shaking for 18 hrs. The residue was washed with acetone (100 ml) and filtered. After pooling, the filtrates were evaporated by distillation under reduced pressure yielding an yellowish mass (2.02 g) in a yield of 1.0 % (w/w).

3. Water- Ethanol (1:1) extract (M-W-EL1299):
The residue (ca. 96 g) was shaken for 18 hrs. at room temperature in 500 ml of a mixture of water, EtOH, 1:1 with 0.5% ascorbic acid. The solvent was filtered and concentrated under reduced pressure at a temperature of 35°C. Above extraction procedure was repeated four times. After concentration, the filtrates were collected and the solvent reduced to one tenth and kept at 2- 4°C for 24 hour. The crystallized matter was filtered and filtrate was kept for another 24 hours at 2-4° C. The crystallized matter was again filtered and taken together to get 1.75 g crystals in a yield of 1.75%. The filtrate was evaporated to dryness to get a solid mass (22.51g) with 1.78 g as crude L- DOPA obtained after crystallization.

4. Chloroform extract (M-CH1299):
100 g of the pulverised seeds of *M. pruriens* were shaken for 18 hrs. at room temperature in 1.7% ammoniated chloroform (300 ml). The extract was filtered and the extraction was repeated three times. The concentrated extract was washed with water (100 ml) and further concentrated to afford 4.0 g extract in a yield of 4.0% (w/w).

5. Ethanol extract (M-EL0100):
20 g of the pulverised seeds of *M. pruriens* were shaken for 18 hrs. at room temperature in EtOH (100 ml). After filtration, the residue was again shaken for 18 hrs. and filtered. The process was repeated for a total of four extractions. The filtrates were concentrated and pooled together to get 1.34 g extract. The extract was tested for the presence of L- DOPA by TLC, positively.

6. Aqueous extract (M-W0100):
The residue obtained from the ethanol extraction, as stated above, was further shaken for 18 hrs. at room temperature in demineralised water and filtered. The extraction was repeated for three times more. The filtrates were pooled together and water distilled off, after passing SO_2 to prevent the oxidation of L- DOPA. The extract (4.68 g) was tested for the presence of L- DOPA.

7. Acetone extract (M-AC0100):
10 g of the pulverised seeds of *M. pruriens* were shaken for 18 hrs. at room temperature in acetone (50 ml). After filtration the residue was again extracted with acetone. The extraction was repeated three times more. The filtrates were pooled together and the solvent distilled off. The extract (0.37g) was tested for the presence of phosphatides.

Example 2: Effect of extracts on primary dopaminergic cultures

Mesencephalic cultures were treated with different concentrations of extracts for 7 days. [^{3}H]Dopamine uptake was measured as an index of dopamine neuron survival and growth. This is a quantitative measurement that reflects the number of dopamine neurons and terminals.

The results of the extracts on native dopaminergic cultures are shown in table 1. Three extracts stimulated at a dose of 0,05µg/ml. However, only one extract, M-PL0100, stimulated at all doses and significantly increased dopamine uptake in the cultures after one week of treatment. The effect was close to a 2-fold increase. However, the SEM (SEM=Structural Equation Modeling, a comprehensive statistical approach to testing hypotheses about relations among observed and latent variables (measured variables and unmeasured constracts) was quite high. Notably, no dose response was observed, possibly due to maximum effectiveness of the lowest concentration used (0.05 µg/ml). M-EL0100 and M-BL0100 showed a significant increase in uptake only at 0.05 µg/ml and LAT543-0 only at 50 µg/ml.

Table 1: Effect of *Mucana pruriens* extracts on the growth of dopamine neurons

	[^{3}H]Dopamine Uptake (% of Control)			
Compound (µg/ml)	**0.05**	**0.5**	**5**	**50**
M-HX1299	124.9 ± 10.2	106.0 ± 11.2	92.9 ± 9.7	100.5 ± 13.3
M-AC1299	115.4 ± 9.0	95.4 ± 8.9	93.1 ± 9.3	121.7 ± 7.1
M-W-EL1299	127.8 ± 10.4	111.1 ± 6.8	109.1 ± 7.5	100.5 ± 12.8
M-CH1299	129.5 ± 18.1	115.8 ± 12.7	105.3 ± 8.1	101.9 ± 9.0
M-EL0100	184.9 ± 20.3*	146.5 ± 14.9	127.8 ± 12.5	140.8 ± 12.5
M-W0100	120.8 ± 9.4	102.7 ± 8.9	103.3 ± 13.3	86.3 ± 11.2
M-ML0100	122.9 ± 13.4	119.5 ± 8.3	115.3 ± 13.1	106.4 ± 10.0
M-BL0100	139.1 ± 19.4*	108.3 ± 9.9	89.4 ± 6.7	130.1 ± 9.3
M-PL0100	206.3 ± 30.4*	187.7 ± 20.3*	170.1 ± 21.3*	191.6 ± 24.5*
LAT543	135.1 ± 26.5	108.6 ± 14.2	117.6 ± 16.3	105.6 ± 10.2
LAT543-0	139.1 ± 19.4	117.9 ± 8.6	120.4 ± 12.4	156.2 ± 14.6*
MACPL0800	-	-	-	-

MWEL0700	-	-	-	-

*p<0.05 ANOVA followed by Dunnett multiple comparison test

Example 3: Effect of extracts in protecting mesencephlic cultures from the toxic effects of MPP^+

Mesencephlic cultures were treated with 5 μM MPP+ for 24 hours in the absence or presence of different concentrations of extracts. [^{3}H]Dopamine uptake was measured 48 hours after removing MPP+ (table 2). Four compounds protected dopamine neurons from MPP+ toxicity (M-W-EL1299; M-W0100 and MWEL0700). All were effective at a concentration of 50 μg/ml.

Table 2: Effect of Mucuna pruriens extracts on the toxicity of MPP+ to dopamine neurons

Extract (μg/ml)	[^{3}H]Dopamine Uptake (% of no MPP+)			
	0	0.5	5	50
M-HX1299	25.7 ± 2.5	27.4 ± 2.6	30.1 ± 3.7	27.3 ± 2.5
M-AC1299	31.4 ± 3.6	37.9 ± 4.4	35.5 ± 3.2	34.9 ± 2.7
M-W-EL1299	25.5 ± 2.5	31.3 ± 3.8	33.2 ± 3.3	63.1 ± 5.7*
M-CH1299	31.3 ± 1.7	32.6 ± 3.1	29.1 ± 3.1	34.3 ± 2.2
M-EL0100	34.6 ± 6.0	33.0 ± 5.3	40.0 ± 5.8	47.2 ± 8.0
M-W0100	39.1 ± 3.7	52.0 ± 4.1	50.8 ± 2.5	59.6 ± 4.6
M-ML0100	28.8 ± 2.0	23.7 ± 1.2	32.9 ± 2.4	50.7 ± 2.4*
M-BL0100	36.0 ± 4.0	32.8 ± 4.6	34.7 ± 4.9	36.5 ± 3.4
M-PL0100	30.6 ± 4.6	31.6 ± 3.9	31.2 ± 4.7	40.4 ± 4.6
LAT543	30.2 ± 3.0	31.8 ± 3.9	34.7 ± 7.2	30.4 ± 4.6
LAT543-0	28.9 ± 1.4	28.3 ± 2.6	27.3 ± 2.1	33.7 ± 1.1
MACPL0800	-	-	-	-
MWEL0700	37.4 ± 2.8	36.4 ± 3.2	49.1 ± 3.4	78.6 ± 8.6*

*Significance p<0.05 or greater; ANOVA followed by Tukey-Kramer multiple comparisons test.

Example 4: Effect of extracts in protecting mesencephalic cultures from the toxic effects of BSO

Mesencephalic cultures were treated with 10 or 50 μM BSO for 72 hours to reduce GSH levels and cause oxidative damage. The extracts were added at the same time as the BSO. The MTT assay was performed to determine cell viability (table 3). As GSH depletion is toxic to all cells, the protection by the extracts in this assay is not necessarily restricted to dopamine neurons.

The LDH assay (table 4) was performed in the medium collected from the cultures and is a measure of cell viability. As this is a non-specific assay, protection by the extracts may not be restricted to dopamine neurons. In the studies, protection against MPP+ toxicity with both the MTT and LDH methods was observed for M-W-EL1299, M-ML0100, and MWEL0700.

Table 3: Effect of Mucuna pruriens extracts on toxicity of GSH depletion (MTT assay)

	MTT Reduction (absorbance at 550)					
BSO (μM)	**0**		**10**		**50**	
Extract (50μg/ml)	–	+	–	+	–	+
M-HX1299	1.60±0.01	1.59±0.03	1.31±0.04	0.23±0.05***	0.76±0.04	0.05±0.01***
M-AC1299	1.55±0.02	1.58±0.02	1.42±0.06	0.15±0.02***	0.83±0.05	0.03±0.00***
M-W-EL1299	1.68±0.11	1.74±0.08	1.52±0.07	1.77±0.09	1.02±0.06	1.81±0.11###
M-CH1299	1.88±0.03	1.95±0.02	1.10±0.12	0.04±0.00***	0.55±0.07	0.01±0.00***
M-EL0100	1.97±0.03	1.95±0.03	1.36±0.02	0.81±0.04*	0.65±0.09	0.11±0.02***
M-W0100	1.26±0.01	1.14±0.04	1.05±0.06	0.70±0.02*	0.66±0.07	0.54±0.05
M-ML0100	1.81±0.05	1.84±0.06	1.48±0.07	1.80±0.05###	0.84±0.08	1.83±0.05###
M-BL0100	1.22±0.03	1.32±0.00	1.17±0.06	0.83±0.12	0.91±0.15	0.12±0.02***
M-PL0100	1.12±0.02	1.07±0.02	1.04±0.02	0.31±0.05***	0.64±0.04	0.03±0.01***
LAT543	1.34±0.05	1.55±0.01	1.20±0.17	0.82±0.16**	0.67±0.16	0.08±0.01*
LAT543-0	1.67±0.02	1.68±0.02	1.24±0.07	0.49±0.03***	0.52±0.05	0.09±0.01***
MACPL0800	1.72±0.03	1.64±0.03	1.20±0.10	0.30±0.04***	0.56±0.10	0.05±0.02***
MWEL0700	1.65±0.03	1.73±0.02	1.04±0.05	1.71±0.02###	0.61±0.03	1.72±0.04###

Table 4: Effect of Mucuna pruriens extracts on toxicity of GSH depletion (LDH assay). The stars in the tables relate to the legends.(p-values).

	LDH released					
BSO (µM)	**0**		**10**		**50**	
Extract (50µg/ml)	–	+	–	+	–	+
M-HX1299	7.8±0.2	8.0±0.4	20.8±0.8	77.9±3.3***	48.0±1.7	75.0±4.6***
M-AC1299	6.9±0.3	6.6±0.2	9.1±0.8	53.7±4.7***	37.1±1.9	60.6±0.6***
M-WEL1299	5.2±0.4	4.9±0.9	11.9±1.7	4.8±0.9***	27.9±5.0	4.8±1.0***
M-CH1299	6.9±0.2	6.4±0.2	37.6±4.7	66.2±1.2***	52.1±2.2	69.5±2.0*
M-EL0100	7.2±0.2	6.4±0.1	28.7±1.2	49.4±0.6***	50.2±1.4	67.7±1.7*
M-W0100	3.4±0.1	5.9±0.6	15.0±2.2	19.4±0.9	27.6±2.4	27.5±2.0
M-ML0100	8.4±0.4	8.0±0.3	20.9±1.9	7.7±0.4***	47.7±2.8	8.7±0.3***
M-BL0100	4.2±0.2	4.6±0.4	8.7±1.1	23.6±4.2**	20.4±4.5	43.9±1.4***
M-PL0100	7.3±0.1	7.4±0.5	10.0±0.5	47.3±2.8***	21.4±5.4	54.8±1.1***
LAT543	3.9±0.2	4.5±0.3	13.6±5.8	29.4±3.9**	37.2±5.6	45.1±1.0
LAT543-0	7.2±0.8	7.5±0.7	28.1±3.9	64.9±2.1***	59.9±2.9	76.3±2.7*
MACPL0800	7.0±0.6	6.0±0.5	31.4±6.2	64.7±1.8***	57.0±5.0	70.8±1.0*
MWEL0700	7.3±0.5	7.3±0.6	41.6±2.1	7.5±0.8***	57.2±1.6	8.9±0.7***

Lactate dehydrogenase (LDH) is a cellular enzyme which is released from damaged or dying cells. Therefore, LDH values increase in case of cellular distress/damage. A strong increase after BSO addition is to be expected to the resulting GSH depletion. Without BSO the extracts did not increase LDH, indicating no toxic effects of the extracts themselfs. With BSO and with increasing dose LDH levels increase strongly with the exception of 3 extracts where LDH is maintained at the o level indicating strong protection. Remarkably, a number of extracts show a rise of LDH levels, indicating increased toxicity. All those extracts contain DMSO which in high concentrations may be toxic. It is likely that in this model DMSO potentiated the BSO toxicity. A similar phenomenon can be seen in table 3.

Something similar may be the case in the stimulation model (table 1). Three extracts stimulate with a dose of 0,05µg/ml. Only M-PLO100 with all doses. All three extracts are alcohol extracts, however, only PL0100 does not contain DMSO and only a trace L-Dopa, the other two extracts contain DMSO and moderate L-Dopa concentration. Thus, it is possible that with increasing doses DSMO and/or L-Dopa (

alone or in synergy) have a toxic effect and thereby undo the stimulatory effect.

Claims

1. A pharmaceutical composition comprising one or more Mucuna pruriens seed components, substances, fractions or mixtures of substances obtained therefrom and a pharmaceutically acceptable diluent, excipient or carrier.

2. The pharmaceutical composition of claim 1, wherein the components, substances, fractions or mixtures of substances are obtained by extraction of Mucuna pruriens seeds.

3. The pharmaceutical composition of claim 2, wherein the components, substances, fractions or mixtures of substances obtained from Mucuna pruriens seed extracts comprise bipolar-lipophilic molecules.

4. The pharmaceutical composition of any one of claims 1 to 3, wherein the composition is formulated as an infusion solution, an injection solution, a gelatin-capsule, a tablet, or a controlled release tablet.

5. Use of Mucuna pruriens seeds or of one or more components, substances, fractions or mixtures of substances obtained or extracted from Mucuna pruriens for the preparation of a pharmaceutical composition for neuroprotection or neurostimulation.

6. Use of one or more Mucuna pruriens components, substances, fractions or mixtures of substances obtained or extracted from Mucuna pruriens for the preparation of a pharmaceutical composition for preventing, alleviating or treating a neurological disease.

7. The use of claim 6, wherein the neuronal disease is a neurological degenerative disease.

8. The use according to claim 7, wherein the neurological degenerative disease is selected from the group consisting of Huntington's disease and Alzheimer's disease or other diseases which are caused by exogenic or endogenic factors.

9. The use according to claim 7, wherein the neurological degenerative disease is Parkinson's disease.

10. The use of any one of claims 5 to 9, wherein the components, substances, fractions or mixtures of substances obtained or extracted from Mucuna pruriens are selected from the group consisting of alkaloids, proteins, peptides, polysaccharides, glycosides, glycoproteins, sterols, phophatids, fatty acids and amino acids.

11. The use of any one of claims 5 to 10, wherein the components, substances, fractions or mixtures of substances isolated from Mucuna pruriens do not contain a pharmaceutically effective amount of L-dopa.

12. The use of any one of claims 5 to 11, wherein at least one alcohol or mixtures of two or more alcohols selected from the group consisting of hexanol, butanol, ethanol, methanol, isopropanol and n-propanol are used for the extraction process.

13. The use of claims 5 to 11, wherein at least one organic solvent or mixtures of two or more solvents selected from the group consisting of chloroform, CO_2 , hypercritical CO_2, ether, DMSO, hexane, ethylacetate, dichlormethane and acetone is used for the extraction process.

14. The use of claims 5 to 11, wherein at least one polar solvent or mixtures of two or more polar solvents selected from the group consisting of water, ethanol, methanol, propanol and isopropanol is used for the extraction process.

15. The use of any one of claims 12 to 14, wherein two or more solvents selected from the group of alcohols, organic solvents and polar solvents used for the extraction process.

16. The use of claim 5 to 15, wherein the extraction is fractionated extraction.

17. A method of preparing extracts or extract-fractions of Mucuna pruriens comprising:

(a) extracting seeds of Mucuna pruriens with n-hexane to provide a first extract solution;

(b) filtering the first extract solution;

(c) extracting the filter retentate of (b) with acetone to provide a second extract solution;

(d) filtering the second extract solution;

(e) extracting the filter retentate of (d) with a 1:1 mixture of water and ethanol containing 0,5% ascorbic acid to provide third extract solution;

(f) filtering the third extract solution;

(g) repeating at least four times the extraction procedure of (e) with the retentate obtained by (f); and

(h) concentrating the pooled extract solutions.

18. A method of preparing extracts or extract-fractions of Mucuna pruriens comprising:

(a) extracting seeds of Mucuna pruriens with an alcohol to provide a first extract solution, wherein the alcohol is methanol, ethanol and/or propanol;

(b) filtering the first extract solution;

(c) repeating at least two times the extraction procedure of (a) with the retentate obtained by (b); and

(d) concentrating the pooled extract solutions.

19. The method of claim 17 or 18, further comprising solubilizing said extract or extract-fractions of Mucuna pruriens obtained in step (h) of claim 17 or

step (d) of claim 18, respectively in a solvent comprising DMSO and/or distilled water.

20. A method for the preparation of extracts or extract fractions of Mucuna pruriens, comprising extracting the seed of Mucuna pruriens with CO_2 or mixtures from CO_2 and butane, propane or other gases under supercritical conditions or different pressures and temperatures, to obtain purification and selection of substances or fractionation of Mucuna pruriens extracts.

21. Use of the extract or extract-fractions of Mucuna pruriens obtainable by the method of any one of claims 17 to 20 for the preparation of a pharmaceutical composition for treating neuronal diseases.

22. The use of any one of claims 5 to 16 or of claim 21, wherein Mucuna pruriens is used in comminuted form, in unmodified form, as granules, powder, precipitate, fraction, extract, dried extract and/or exudate, preferably as extract.

23. The use of any one of claims 5 to 16 or of claim 21, wherein one or more of the Mucuna pruriens components, substances, fractions or mixtures of substances obtained therefrom are used in combination with one or more other active agents.

24. The use of any one of claims 5 to 16 or of claim 21, wherein the Mucuna pruriens components, substances, fractions or mixtures of substances are formulated as infusion solution, injection solution, for oral forms of application, as a therapeutic pack, a granulate, a food supplement or in form of clysters.

25. The use of any one of claims 5 to 16 or of claim 21, wherein the application is oral application, topical application or parenteral application.

26. A kit comprising one or more containers filled with Mucuna pruriens components, substances, fractions or mixtures of substances or the pharmaceutical compositions of claim 1 to 4.

Abstract

The present invention provides pharmaceutical compositions comprising Mucuna pruriens seeds or one or more Mucuna pruriens seed components, substances, fractions or mixtures or substances obtained therefrom. Furthermore, the invention relates to the use of Mucuna pruriens seed powder or one or more Mucuna pruriens components, substances, fractions or mixtures or substances obtained therefrom for the preparation of a pharmaceutical composition for preventing, alleviating or treating neurological diseases. Additionally, the invention relates to the use of Mucuna pruriens seeds for the preparation of a pharmaceutical composition for neuroprotection or neurostimulation and to methods of preparing extracts of Mucuna pruriens which can be used for the preparation of a pharmaceutical composition for treating neurological diseases.